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The impact of sediment bioturbation by secondary organisms on metal bioavailability, bioaccumulation and toxicity to target organisms in benthic bioassays: implications for sediment quality assessment

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Abstract
Bioturbation alters the properties of sediments and modifies contaminant bioavailability to benthic organisms. These naturally occurring disturbances are seldom considered during the assessment of sediment quality. We investigated how the presence (High bioturbation) and absence (Low bioturbation) of a strongly bioturbating amphipod within three different sediments influenced metal bioavailability, survival and bioaccumulation of metals to the bivalve Tellina deltoidalis. The concentrations of dissolved copper decreased and manganese increased with increased bioturbation. For copper a strong correlation was observed between increased bivalve survival (53-100%) and dissolved concentrations in the overlying water. Increased bioturbation intensity resulted in greater tissue concentrations for chromium and zinc in some test sediments. Overall, the results highlight the strong influence that the natural bioturbation activities from one organism may have on the risk contaminants pose to other organisms within the local environment. The characterisation of field-based exposure conditions concerning the biotic or abiotic resuspension of sediments and the rate of attenuation of released contaminants through dilution or readsorption may enable laboratory-based bioassay designs to be adapted to better match those of the assessed environment.

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The impact of sediment bioturbation by secondary organisms on metal bioavailability, bioaccumulation and toxicity to target organisms in benthic bioassays: implications for sediment quality assessment

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Highlights:

- Bioturbation intensity modifies metal exposure and outcomes of sediment bioassays.
- Sediment fluxes of Cu decrease and Mn and Zn increase with increased bioturbation.
- Strong correlations between bioaccumulated and dissolved Cd, Cr, Pb, Zn, Cu and Ni.
- Weak correlations between bioaccumulated and particulate metals.

Capsule Abstract

This study investigated the impact of sediment bioturbation intensity on metal bioavailability and toxicity to aquatic organisms, and the implications of this to toxicity test design.

Abstract:

Bioturbation alters the properties of sediments and modifies contaminant bioavailability to benthic organisms. These naturally occurring disturbances are seldom considered during the assessment of sediment quality. We investigated how the presence (High bioturbation) and absence (Low bioturbation) of a strongly bioturbating amphipod within three different sediments influenced metal bioavailability, survival and bioaccumulation of metals to the bivalve *Tellina deltoidalis.* The concentrations of dissolved copper decreased and manganese increased with increased bioturbation. For copper a strong correlation was observed between increased bivalve survival (53 to 100%) and dissolved concentrations in the overlying water. Increased bioturbation intensity resulted in greater tissue concentrations for chromium and zinc in some test sediments. Overall, the results highlight the strong influence that the natural bioturbation activities from one organism may have on the risk contaminants pose to other organisms within the local environment. The characterisation of field-based exposure conditions concerning the biotic or abiotic resuspension of sediments and the rate of attenuation of released contaminants through dilution or readsorption may enable laboratory-based bioassay designs to be adapted to better match those of the assessed environment.

Keywords: Bioassays, benthic invertebrates, contaminants, speciation, sediment quality guidelines

1.0 Introduction

The degree to which organisms modify sediments is influenced by sediment properties, biogenic activity (burrowing, feeding behaviour etc), and the frequency of sediment reworking. This is clearly shown by studies with amphipods (*Corophium volutator*), polychaete worms (*Lumbriculus variegatus, Arenicola marina*, *Nereis diversicolor*, *Nereis virens*, *Heteromastus filiformis* and *Tubifex tubifex*), bivalves (*Tellina texana*, *Macoma balthica* and *Cyclope neritea*), and sea cucumbers (*Holothuria whitmaei*) (Aller and Yingst, 1985; De Backer et al., 2011; Lagauzère et al., 2009; Peterson et al., 1996; Pischedda et al., 2008; Shiell and Knott, 2010; Volkenborn et al., 2010). Community structure and population density are further factors which influence the extent of sediment disturbance by organisms (Forbes, 1994; Thrush et al., 2006; Wethey et al., 2001).

Metal(loid) bioavailability and the rate of metal accumulation by benthic organisms is influenced by biokinetics, organism behavior and physiology, and sediment chemistry, particularly factors that impact the partitioning between aqueous and solid phases and metal speciation in these phases (Fe/Mn (oxy)hydroxides and DOC) (Costello et al., 2015; Fisher et al., 1980; Simpson and Batley, 2007). The bioturbation of sediments by large benthic invertebrates alters sediment redox chemistry by mixing pre-stratified zones in the sediment, and increasing the penetration of electron acceptors such as dissolved O_2, NO_3^- and SO_4^{2-} into anoxic sediments (Aller et al., 2001; Granéli, 1979; Matisoff et al., 1985; Pischedda et al., 2008; Volkenborn et al., 2010). Redox changes can alter metal binding affinities between the solid and dissolved phases, significantly modifying the speciation and bioavailability of most metals in sediments (De Jonge et al., 2012; Doyle and Otte, 1997; Granberg et al., 2008). The concentration of AVS has a major influence on metal bioavailability, and for sediments containing a molar excess of acid volatile sulfide (AVS) over simultaneously extractable metals (SEM, ΣCd, Cu, Ni, Pb, Zn), it is predicted that the porewater concentrations of these metals will be negligible and should not cause direct toxicity to benthic organisms (Ankley et al., 1996; Hansen et al., 2005; Lawrence et al., 1982). In addition, the activity of microbes such as *Desulfuromonadales* and *Geobacter sulfurreducens* in abandoned burrows also contributes to the release of metals from anoxic sediments to the pore waters and overlying water column (Kristensen, 2008; Meysman et al., 2006). Thus bioturbation processes can modify the exposure and risk posed by contaminants to the organisms and surrounding ecosystem (Atkinson et al., 2007; Ciutat and Boudou, 2003; Simpson et al., 2002).

Within sediment quality assessments programs, laboratory-based bioassays are frequently used to assess bioaccumulation and toxicity (ASTM, 2014, 2010). To prevent predation by indigenous organisms on the test species, methods generally specify the removal of large indigenous organisms before testing. While the exposure conditions in laboratory bioassays need not exactly resemble field conditions, the bioassays should aim to provide assessment outcomes that would be similar to those of sediments that remained in their natural field setting. In this study we hypothesize that the bioturbation activities of indigenous organisms may be sufficient to influence the outcomes of bioaccumulation and toxicity assessments, i.e. whether effects are detected, and potentially alter the outcome of assessment programs.

The specific objectives of this work were therefore to: 1) investigate how different bioturbation intensities alter the bioavailability, bioaccumulation and toxicity of metals, and

2) evaluate the influence that bioturbation by secondary organisms may have on the outcomes of sediment bioaccumulation and toxicity bioassays. The three different sediments were subjected to the presence of no organisms, a benthic bivalve (*Tellina deltoidalis*; low bioturbation), and this bivalve combined with a highly bioturbating amphipod (*Victoriopisa australiensis*; high bioturbation) and the metal bioaccumulation and toxicity (survival) to the bivalve was assessed. Changes in metal bioavailability were assessed through measurements of AVS, dissolved metal release to overlying water and bioaccumulation by the bivalve. Differences in metal bioaccumulation for the Low and High bioturbation treatments are discussed in relation to the observed differences in metal bioavailability and how assessment outcomes might be modified by varying degrees of bioturbation.

2.0 Material and Methods

2.1 General Methods

All chemicals used were AR grade or equivalent analytical purity. Deionised water (18 MΩ.cm, Millipore) was used for all solutions. Glass and plastic consumables used for analysis were new and acid-washed via soaking in 10% (v/v) HNO_3 (>24h, BDH, AR Grade), followed by thorough rinsing with deionised water. Glass beakers used for bioassays were washed in a dishwasher (Gallay Scientific) with detergent followed by acid-washing (5% HNO_3 (v/v)) and rinsing using reverse-osmosis purified water.

Physicochemical parameters of dissolved oxygen (DO), temperature, salinity and pH were routinely measured using WTW instruments (Wissenschaftlich-Technische Werstätten) calibrated according to manufacturer's instructions: an Oxi 330 Oximeter, LF320 Conductivity meter, and a pH 320 meter. Dissolved ammonia was measured using a rapid test kit (API Fish Care, LR8600). Overlying water samples were immediately filtered (0.45 µm cellulose nitrate, 25 mm, Minisart, Sartorius) and acidified to 2% HNO_3 (v/v, Tracepur, Merck). Total suspended solids (TSS) were determined gravimetrically; overlying water samples (350 mL) were filtered (cellulose nitrate ester filters, 0.45µm, *in vacuo* Sartorius polycarbonate filter rig), dried (70°C, 48 h), and weighed.

Subsamples of homogenised bulk sediment were collected prior to sediment transfer into test vessels, and at the end of the study as mini-cores (1 cm diameter, 4 cm depth; 10 mL, polycarbonate vials) from each test vessel. The subsamples and mini-cores were immediately frozen (-20°C) until analysis. The gravimetric determination of the dry:wet sediment ratio (D:W) and fine sediment fraction (<63 µm) was conducted as per Belzunce-Segarra et al. (2015). Total recoverable metals (TRM) were determined after low-pressure microwave-assisted (MARS 5, CEM) aqua regia digestion (3:1 HNO_3:HCl). Total organic carbon (TOC) analysis was conducted using a CO_2 evolution method. Dried and crushed samples were acid-treated to remove inorganic carbonates followed by high temperature combustion (LECO furnace) in the presence of strong oxidants/catalysts using infrared detection. Within a nitrogen filled glove box, the frozen sediment mini-cores were extruded and sections taken from the surface (~1.5 cm from the top) and at depth (~1.5 cm from the bottom) for analyses of AVS and AEM (Simpson, 2001).

Biological tissues (~0.1 g) were freeze-dried (24 h, Christ Freeze Drier), digested in a HNO_3:H_2O_2 digestion solution (2 mL concentrated HNO_3 (Tracepur, Merck): 1 mL concentrated H_2O_2 (Merck) for 18 h at 25°C) and microwave-heated for 1 h (MARS 5, CEM, programmed RT- 60°C, 12 min; 60 – 65°C, 10 min; 65- 70°C, 10 min; 70°C for 10 min) before a 10-fold dilution using deionised water for metal analysis.

Metal analyses in waters and acid digests were performed on a combination of inductively coupled plasma – atomic emission spectrometry (ICP-AES, Varian 730-ES) and inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500ce). For the purpose of QA/QC, 10% of all samples analysed were blanks and 30% were duplicates. Certified reference material recoveries for both sediment (ERM®-CC018, European Reference Materials (ERM)) and biological tissues (DORM-3, *Mytilus galloprovincialis,* NRCC), were analysed with the respective samples and were within 75-125% of expected values. The limits of reporting for the various methods were less than 10% of the lowest measured values.

2.2 Test Media and Organisms

Clean seawater sourced from the southeast coast of New South Wales (NSW), Australia, was filtered (1 µm) and analysed (ICP-AES) before use to ensure that metals of interest were below 1 µg L^{-1}. Clean and contaminated sediments (0-15 cm depth) were collected from Lake Illawarra, Port Kembla, Bonnet Bay and Kings Bay, NSW. Sediments were sieved on-site (2 mm plastic mesh) to remove coarse material (e.g. detritus and leaves) and any large fauna, then thoroughly homogenised and stored in polyethylene bags at ~4°C in the dark. Lake Illawarra (S1) and Kings Bay (S3) sediments were used unmodified, whereas sediment from Port Kembla was mixed 1:1 with Bonnet Bay sediment to achieve the desired contaminant concentrations (S2) as discussed by Belzunce-Segarra et al. (2015).

The deposit-feeding amphipods *Victoriopisa australiensis* (Chilton, 1923) (2-3 cm body length) were collected from Lake Illawarra (34°3'S, 150°49'E), a large coastal estuarine system. *V. australiensis* inhabits estuarine, littoral, mud flats and seagrass sediments of south-eastern Australia (Dunn et al., 2009; Lowry and Springthorpe, 2005). The amphipod inhabits fixed burrows, and feeds on subsurface sediments as they excavate and redeposit the detritus back into their burrows. The deposit-feeding bivalve *Tellina deltoidalis* (Tellinidae; Lamarck 1818) (1.5-2 cm shell length) was collected from the estuarine mud flats of the Lane Cove River, adjacent to Boronia Park, Hunters Hill, NSW (33°49'S, 151°8'E). *T. deltoidalis* inhabits estuarine and coastal lagoon sediments of south-eastern to south-western Australia (King et al., 2010, 2004; Wethey et al., 2001). Organisms were collected and maintained in the laboratory prior to tests as described by King et al. (2004).

2.3 Bioturbation Bioassays

The bioassays were undertaken in 1-L chambers (glass beakers, D = 145 mm) with a sediment depth of 5.5 cm depth (400 mL, 550-650 g of wet sediment) and 700 mL of filtered seawater (32 ± 2 PSU). The overlying waters were aerated for 14 d before tests commenced, with three water changes per week to allow the sediments to equilibrate. For each of the three sediments, three treatments were prepared in triplicate (i.e. 27 chambers): controls with no organisms (No), low bioturbation using bivalves (Low), and high bioturbation using

bivalves plus amphipods (High). Five bivalves were added to the Low and High treatments and 6 amphipods were added to the High treatments.

All chambers were maintained at ~ 21°C for 28 d under ambient laboratory light conditions and supplied with aeration throughout the tests. The overlying waters were renewed every two days, and sampled for dissolved metal analyses 3 times per week immediately prior to water renewal. Water quality measurements (temperature, DO, pH and total ammonia (maintained <2 mg NH_3-N L^{-1}) were also performed prior to water renewals (Supplemental Figure A1). At test completion (day 28), sediment cores were collected, and organisms were retrieved (coarse sieve), allowed to depurate for 24 h (21°C) in clean filtered seawater (0.2 µm, Millipore filter), and chilled (1-4°C, 2 h) to anaesthetize. *T. deltoidalis* soft tissue mass (extracted using a clean Teflon-coated blade) and *V. australiensis* (whole) were stored in pre-acid-washed vials and immediately frozen.

2.4 Statistical Analyses

Metal concentrations in *T. deltoidalis* exposed to different types of sediments and bioturbation intensities (Low and High) were tested for statistical differences using two-way analysis of variance (ANOVA) with interactions, followed by Tukey's test. Normality of residuals (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) were tested prior to hypothesis testing. When either residuals were not normally distributed or data were heteroscedastic, Kruskall-Wallis' test was applied to investigate statistical differences. Statistical analysis was conducted using the software R 3.12 (x64). Unless otherwise stated, α=0.05 was the level of significance.

3.0 Results and Discussion

3.1 Sediment Properties

The physicochemical properties and concentration of major metal contaminants, as total recoverable metals (TRM) and dilute-acid extractable metals (AEM), are summarised in Table 1. Additional data for other significant metal(loid)s, along with TRM concentrations of the <63 µm sediment fraction are provided in Table A1 in the Supplementary Information. The metal contamination within the sediments ranged from relatively clean (S1) to highly contaminated (S2 and S3).

The S1 and S3 were both sandy sediments, with low TOC (~1%). S1 had negligible AVS and SEM-AVS (0.1 and 0.5 µmol g^{-1}, respectively). S3 had high AVS and moderate SEM-AVS (10 and 4.3 µmol.g^{-1}, respectively). S2 was silty with a higher TOC (6%), low AVS and relatively high SEM-AVS (0.2 and 13 µmol.g^{-1}, respectively). With respect to the normalized excess SEM (SEM-AVS)/f_{OC}) data, the concentrations increased in the order 45 (S1), 230 (S2), and 360 (S3) µmol.g^{-1}.

The total recoverable metal (TRM) concentrations in S1 were below the sediment quality guideline (SQG) values. In S2 the total recoverable (TR)-copper, -lead and -zinc concentrations (550, 460 and 900 mg kg^{-1}) were above the SQGs of 65, 50 and 200 mg kg^{-1}, respectively, and in S3 were >10× the SQGs (1200, 1400 and 2800 mg kg^{-1}, respectively). TR-chromium in S3 (390 mg kg^{-1}) also exceeded the SQG of 80 mg kg^{-1}. The AEM/TRM ratio provides an indication of the more labile, and potentially bioavailable, fraction of each metal

within the sediment. For copper, lead and zinc, the AEM forms accounted for 50-81% of the TRM in S1, 40-76% in S2 and 0.3-30% in S3 sediments. The very low portion of AE-Cu measured in S3 was attributed to the high AVS content of the sediment, and the low solubility of copper sulfide phases in 1 M HCl (Simpson et al., 1998).

3.2 Organism Behaviour and Responses

The bivalve was observed to exhibit avoidance behaviour in tests containing S2 (for ~20% of test organisms) and S3 (~30-40% of test organisms) sediments. These observations included delayed burrowing, prolonged testing of surrounding sediment using syphons before burial, and relocation attempts by using their 'foot' to flip around to find more favourable sediment conditions. Where burial did not occur within 4 h, pilot holes (2 cm deep) were made with a spatula to facilitate burrowing (~10% (S2) and 25-30% (S3) of all organisms). Avoidance-type behaviour has been documented for a range of organisms in response to metal-contaminated sediment, including bivalves *Scrobicularia plana* and *Macomona liliana* (Kalman et al., 2015; Lefcort et al., 2004), amphipods, copepods and snails (Ward et al., 2013). Avoidance behaviour was not evident for *V. australiensis* which had generated burrows within 10 min of exposure to all sediments.

One bivalve was replaced with another of similar size on Day 0 in one replicate of sediment S3 after the bivalve failed to burrow within 2 h of being provided a pilot hole. Three dead amphipods (one on Day 2 and two on Day 14) were replaced in S3. At the completion of the 28-day tests, 100% survival of bivalves was observed for the Low and High bioturbation treatments in sediment S1, and amphipod survival was 89% in the High treatment. In sediments S2 and S3, bivalve survival (mean ± SE) was 53 ± 0.4 % and 87 ± 0.6 % in Low bioturbation treatments, respectively, and 100% in High bioturbation treatments. Amphipod survival was 83 ± 0.4 % in S2 and 94 ± 0.2 % in S3 (mean ± standard error). Bivalve and amphipod survival data are provided in Table A2 (Supplementary Information).

The bioturbation activity of the bivalves and amphipods had very distinct and contrasting impacts on the sediment structure and the turbidity within the water column. Photographs showing the sediment profiles and water column are provided in Figures S2 and S3 of the Supplementary Information, and illustrate the size and shape of burrow structures and suspended solids in the overlying water. The TSS concentrations differed significantly between treatments, and increased with bioturbation intensity from the No < Low << High bioturbation (Table 2, Figure A2). Differences in the amount of fine particles in the three sediments contributed to these observations (Table 1), with the mean TSS concentration for the silty-S2 High bioturbation treatment (76% <63 µm particles) being more than twice those measured for the more sandy S1 and S3 sediments (both ~30% <63 µm). The changes in metal bioavailability as a consequence of the differing amounts of fine sediment re-suspension and dispersal due to bioturbation were therefore influenced by both sediment properties (abiotic processes) and organism behaviour (biotic processes) (Meysman et al., 2006; O'Shea et al., 2012).

3.3 Sediment Metal Bioavailability

Owing to the significance of sulfide as a binding phase for the metals Cd, Cu, Ni, Pb and Zn, the potential for oxidation of AVS in surface sediment is an important consideration when evaluating metal bioavailability, and may be facilitated/ enhanced by bioturbation (De Lange et al., 2006; Peterson et al., 1996; Simpson et al., 2012). For sediments S1 and S2, the AVS concentrations in the initial unexposed sediments were ≤0.2 μmol g^{-1}, and increased to a maximum of 2 μmol g^{-1} observed in the S1 surficial and deep layers by 28 days (Table 2). For S3 sediment, AVS concentrations were higher in the surficial than the deeper sediment in No and High bioturbation treatments, but the reverse in the Low treatments. Bioturbation-driven changes to sediment SEM and SEM-AVS concentrations are displayed in Figure 1. The SEM concentrations were higher in treatments with High bioturbation than those with No bioturbation in surficial S1 sediments and both surficial and deeper S2 sediments; however, for S3 deep sediments, SEM was lower in tests with High bioturbation. No bioturbation-related trends for SEM were observed at depth in S1 sediments or in surficial S3 sediments. The molar differences between SEM and AVS concentrations (SEM-AVS) were greater in treatments with High bioturbation, compared to the No bioturbation treatment in all surficial and depth sediments, except for deep S3 which had the higher AVS concentrations. Overall, the SEM-AVS model would predict that increased bioturbation would significantly increase the bioavailability of metals in the surface sediments and the risk posed to organisms whose surface sediments are the dominant metal exposure route. There were no clear relationships between survival of either organism and SEM or SEM-AVS concentrations measured at the start or completion of the experiments.

Typically, a significant portion of the TRM will not be bioavailable to organisms, and the AEM concentrations (which is equivalent to SEM) frequently provides a more useful estimate of the 'potentially bioavailable' metal concentration. Differences in bioturbation intensity induced changes in the AEM concentrations of AE-manganese, -copper and -zinc (Table 2, and Supplementary Information Tables A5-A6). In the surface sediments, the AEM concentrations were frequently higher in sediments exposed to higher bioturbation than those with No organisms in surficial sediments, but was the opposite for deeper sediments. For AE-copper, there appears to be a higher degree of heterogeneity, and this was attributed to varying degrees of oxidation of copper sulfide phases, that once oxidised become extractable in 1 M HCl (Simpson et al., 1998). In S2 sediments, the order of AEM concentrations for manganese, copper and zinc were No < Low < High for manganese, No < Low << High for copper and No << Low << High for zinc in tests with No, Low and High bioturbation, respectively. At depth, the order of AEM concentrations for manganese, copper and zinc were No < Low=High for manganese, No < Low < High for copper and No < Low < High for zinc, respectively. Overall, the observed bioturbation facilitated increased concentrations of bioavailable metals (AEM) in the surface sediments relative to the deeper sediments.

3.4 Overlying Water Chemistry

The dissolved concentrations of cadmium, copper, manganese, nickel, lead and zinc in the overlying water during the bioassays are shown in Figure 2, with data for other metals and TSS concentrations provided in Table 2 and Tables A3 and A4 of the Supplementary Information. Dissolved copper concentrations were generally lower, and dissolved

manganese concentrations higher, in treatments with greater bioturbation (Figure 2, Table 2). This was most evident for sediment S3 where the mean dissolved metal concentrations in the overlying water were 15, 9.1 and 0.8 µg L^{-1} for copper and 1, 11 and 49 µg L^{-1} for manganese in the No, Low and High bioturbation treatments, respectively. Bioturbation resulted in significant increases in dissolved zinc concentrations in S3 but decreases in dissolved zinc in S2 (Table 2, Figure 2). Only for sediment S3 were there substantial bioturbation-induced changes in overlying water concentration for cadmium, nickel and lead. Dissolved cadmium and lead (during the first 10 days only) were lower in sediments exposed to High bioturbation compared to those with No bioturbation, where the mean dissolved cadmium and lead concentrations in overlying waters were No > Low > High and No = Low > High bioturbation, respectively. As with zinc, dissolved nickel increased with increasing bioturbation in S3.

The patterns of metal release appeared to be largely uninfluenced by the water changes that were made 3 times per week (as indicated in Figure 2). For sediment S3 there were generally increasing releases of dissolved cadmium, nickel and zinc during both the Low and High treatments and for copper during the Low treatment. In contrast, zinc concentrations were steady or dropping during the treatments for S2. For manganese, there was an initial rapid release and then concentrations generally decreased during the treatment which will have been influenced by the rate of resupply of manganese in the pore waters after the initial release due to bioturbation (very little manganese was release in the No bioturbation treatment (natural diffusive release) for any sediment).

The lower dissolved copper concentrations in the High bioturbation treatments are indicative of increased copper adsorption to the newly formed Fe/Mn (oxy)hydroxide phases that were expected to have precipitated at the sediment-water interface and were being resuspended into the overlying water column through bioturbation. This is supported by the higher amounts of dissolved manganese and suspended particulates in the overlying waters for the High bioturbation treatments (Table 2). Greater concentrations of dissolved Mn(II) compared to Fe(II) is attributed to the slow oxidation kinetics of Mn(II) in seawater, whereas Fe(II) is rapidly oxidized and reprecipitated as the corresponding iron (oxy)hydroxide phases (Glasby and Schulz, 1999).

The increases in dissolved cadmium, nickel and zinc concentrations observed during the experiments in S3 treatments were attributed, in part, to the higher initial AVS concentrations in this sediment. Decreases in AVS concentrations were considerable for the surface sediments of S3 (Table 2), and although the rates of oxidation differs considerably for cadmium, copper, nickel, lead and zinc sulfide phases (Ankley et al., 1996; Di Toro et al., 1990; Simpson et al., 1998), the sulfide phases of all of these metals would be expected to oxidise to some degree during the 28-day experiment. A significant fraction of the metals released from the sulfide phases would be re-adsorbed to other sediment phases (Burdige, 2006; Smart et al., 2003) or be released to the overlying waters (Cantwell et al., 2002). Based on previous studies of metal adsorption to iron and manganese (oxy)hydroxide phases, copper and lead have greater sorption affinities for Fe/Mn hydrous oxides than zinc and nickel (Davis and Leckie, 1978; Schwertmann et al., 2000).

3.5 Relationship between Tissue Metal Concentrations and Exposure

In general, the final bivalve tissue concentrations of cadmium, chromium, nickel, lead and zinc was greatest from sediment S3, while the concentrations of these metals in bivalve tissues were similar for sediments S1 and S2 (Figure 3). The tissue concentrations of arsenic and copper were greater from sediments S2 and S3 than S1. The tissue concentrations of manganese were greater from sediment S1 than S2 and S3. Manganese tissue concentrations in amphipods decreased with increasing sediment manganese concentrations, in the order of S1>S2>S3.

The Low and High bioturbation treatments had quite variable influences on the bivalve tissue metal concentrations (Figure 3). No significant differences were observed between Low and High bioturbation treatments for arsenic or cadmium in any sediment. For sediments S2 and S3, higher tissue concentrations of chromium ($p=0.01$, S3) were observed in bivalves exposed to High bioturbation than those in Low bioturbation treatments, but there was no difference between High and Low treatments in S1 ($p > 0.5$). The final tissue copper concentration of bivalves from S2 sediments was greater when exposed to Low rather than High bioturbation, and this was consistent with the greater copper release occurring in the Low bioturbation treatments (Figure 2, Table 2). However, there was no statistically significant difference in bivalve tissue copper concentrations observed with differing degrees of bioturbation in S1, S2 and S3 sediments, despite the considerably higher mean dissolved copper concentrations in the Low bioturbation treatments (9 ± 3 (Low) and 1 ± 1 μg L^{-1} (High) S3). Final tissue concentrations for manganese appeared to be higher in bivalves exposed to Low bioturbations compared with those exposed to High bioturbation in sediment S1, but was opposite in S3 sediments. However, these are statistically inconclusive, most likely due to the low number of replicates treatments (n=3). There was no significant difference in manganese accumulation between treatments observed for S3. The final tissue concentrations of nickel in the bivalves were variable across all three sediments (Figure 3). For both lead and zinc, final tissue metal concentrations were generally greater in High bioturbation treatments, but significant differences ($p=0.05$) were only observed in S3 for zinc.

The linear relationships between metal concentrations in the bivalves and the dissolved and particulate metal concentrations were investigated (Figure 4, Figures A4-A6 of the Supplementary Information). There were strong relationships between tissue metal concentrations and dissolved metal concentrations in the overlying waters for cadmium (R^2= 0.79), chromium (R^2= 0.84), lead (R^2= 0.72) and zinc (R^2= 0.88) and tangible relationships for copper (R^2= 0.48), nickel (R^2= 0.62), but generally weaker relationships between tissue metal concentrations and AEM (Figures S5 and S6). Lee and Lee (2005) found similar strong relationships between chromium assimilation by the Asiatic clam *Corbicula fluminea* from the dissolved phase although they observed weaker correlations for particulate phase assimilation.

The bivalve, *T. deltoidalis*, is endobenthic, using its siphon to feed on particles at the sediment:water interface and also filter particles from overlying water (Campana et al., 2013). A biokinetic model of copper bioaccumulation exists for the bivalve (King et al., 2005), and for sediment S2 (with the highest AE-copper concentrations) the exposure to particulate copper through ingestion of fine sediments while foraging for food would be predicted to be a major source of accumulated copper. In sediment S3 the AE-copper concentration was very low at the start, but higher and quite variable at the completion of

the experiments (Table A5) and may also have contributed to the observed copper accumulation in the bivalves in this sediment (Figure A6). Weak relationships were between bivalve copper concentrations and dissolved copper (r^2 = 0.48, Figure 4) and the mean of initial and final AE-Cu concentrations (r^2 = 0.52, Figure A6). While these relationships do not indicate which of dissolved or particulate copper contributed more to the bivalves copper exposure, together with the high AE-Cu (280 mg/kg), the higher dissolved copper concentration in the Low bioturbation treatments for sediment S2 may have been the 'tipping point' resulting in the decreased bivalve survival in that sediment treatment. In addition, the dissolved copper concentration was also higher during the Low bioturbation treatment of sediment S3 (9 µg/L), but the AE-Cu concentration remained relatively low (<10 mg/kg).

The observed positive relationships between bivalve tissue lead concentrations and dissolved lead concentrations, but not sediment-lead concentrations, is consistent with the dissolved lead exposure resulting in lead accumulation. The opposite was observed by Belzunce-Segarra et al. (2015), who found positive bioaccumulation in *T. deltoidalis* and sediment lead concentrations, but no correlation with dissolved lead concentrations, indicating particulate lead to be likely to have enhanced lead accumulation. In both studies the relationships are quiet weak and highlight the difficulty in assessing metal exposure routes using small data sets in which a few data points can strongly influence the strength and direction of any relationships. The studies by King et al. (2005) observed that zinc bioaccumulation is generally well explained by the dissolved zinc concentrations. For manganese, the bioaccumulation observed in S1 was much greater than the S2 and S3 sediments, and neither the dissolved nor the particulate manganese concentrations provided a useful relationship with the observed manganese bioaccumulation. The manganese bioaccumulation did decrease with increasing (SEM-AVS)/f_{OC}, indicating a possible response to increased concentrations of bioavailable metals (cadmium, copper, nickel, lead and zinc), however the data set was not large enough to speculate further on this (as organisms may have been healthier in S1, and/or had different rates of filtering than other organisms etc.).

3.6 Implication for Sediment Quality Assessment

Sediments that release greater concentrations of metals into overlying waters, whether in dissolved or particulate form, are expected to pose a greater risk of bioaccumulation and toxicity to both benthic and aquatic organisms (Birch and Hogg, 2011; Simpson et al., 2012; Amato et al., 2014; Campana et al., 2013). Metal fluxes are exacerbated by sediment-organism interactions, particularly the degree/intensity of bioturbation which is dependent on organism type, population density and sediment composition (particle size, partitioning, AVS and TOC content) and the properties, concentration and form of the metal (Peterson et al., 1996; Pischedda et al., 2008; Volkenborn et al., 2010).

This study has affirmed bioturbation intensity to be an important vector for altering metal bioavailability and exposure to benthic organisms and may change the outcomes of sediment bioaccumulation and toxicity assessments. Due to the expected greater dilution of contaminant fluxes in the field, the use of laboratory-based bioassays will have exaggerated the influence of bioturbation in the present study. Nevertheless, the magnitude of

sedimentary disturbance which occurs in laboratory-based bioassays will generally be miniscule compared with those experienced in the natural environment, especially where larger bioturbating organisms are present (O'Shea et al., 2012).

The difficulties associated with extrapolating test outcomes from laboratory-based bioassays to conditions experienced in the natural environment is well documented (Burton et al., 2005; 2012; Mann et al., 2010, Belzunce-Segarra et al., 2015), as is the difficulty of designing robust field-based tests. Observations of both biotic (e.g. presence of other organisms) and abiotic (e.g. sediment resuspension due to water currents) conditions at the location(s) where sediments being assessed are collected will assist in improving the design of laboratory-based bioaccumulation and toxicity tests. Components of the laboratory designs may benefit from being adaptable, to allow exposure conditions, such as those influenced by bioturbation intensity, to better match the assessed environment and enable the impact of these generally excluded natural conditions to influence assessment outcomes.

These conclusions also further emphasise the benefits that may be gained from well-designed field-based tests (Belzunce-Segarra et al., 2015; Burton et al., 2005; Burton et al., 2012; Liber et al., 2007) which may be deployed in zones where processes such as bioturbation by non-target organisms can naturally influence contaminant bioavailability and exposure.

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Tables and Figures for the Main Text

Table 1 Physicochemical properties of initial sediments used for the 28-day bioturbation bioassay

Sediment	Fe	Mn	Cd	Cr	Cu	Ni	Pb	Zn	<63 µm	TOC	AVS
	(%)	Total recoverable metals (TRM) (mg kg^{-1} or % for Fe, dry weight)							%	%	µg mol^{-1}
S1	0.8 ± 0.1	44 ± 16	0.3 ± 0.1	5.0 ± 1	16 ± 2	2.3 ± 0.3	10 ± 1	37 ± 4	32 ± 2.2	1.1	0.1 ± 0.03
S2	4.0 ± 0.5	240 ± 90	2 ± 0.2*	68 ± 1	550 ± 10+	19 ± 0.1	460 ± 6+	900 ± 3+	76 ± 0.4	5.7	0.2 ± 0.1
S3	0.5 ± 0.02	67 ± 2	9 ± 0.1*	390 ± 10+	1200 ± 30+	36 ± 0.7*	1400 ± 20+	2800 ± 30+	27 ± 3.1	1.2	10 ± 0.7
	Dilute-acid extractable metals (AEM) (mg kg^{-1} or % for Fe, dry weight)								SEM	SEM-AVS	SEM-AVS/ f_{OC}
									(all in µmol g^{-1})		
S1	0.3 ± 0.09	33 ± 6	0.2 ± 0.05	1.0 ± 0.1	7.0 ± 0.4	1.0 ± 0.04	8.0 ± 0.2	30 ± 1	0.6 ± 0.01	0.5 ± 0.01	45
S2	1.0 ± 0.2	113 ± 14	1.0 ± 0.2	24 ± 2	240 ± 20*	5.6 ± 0.3	380 ± 20+	560 ± 30+	13 ± 1	13 ± 0.6	230
S3	0.4 ± 0.06	25 ± 3	2.0 ± 0.2*	55 ± 6	0.4 ± 0.04	5.0 ± 0.5	390 ± 60+	850 ± 94+	14 ± 2	4.3 ± 2.1	360
Guideline Value (SQGV)			*1.5*	*80*	*65*	*21*	*50*	*200*			
SQGV – high			*10*	*370*	*270*	*52*	*220*	*410*			

<63 µm (%) = percentage (by mass) of fine sediment particles; AEM = 1 M HCl extractable metals. AVS = acid-volatile sulfide. The Sediment Quality Guideline Value (SQGV) and SQGV-high are obtained from the recommended revised ANZECC/ARMCANZ sediment quality guidelines (Simpson et al., 2013). * = exceed Guideline Values, + = exceed SQGV-high guidelines. Data for Al, Ag, As and V can be found in Table A1 of the Supplementary Information. Data for TOC are derived from single samples (n=1), the accuracy of the method was validated to be within ± 30% of the certified reference material (NEPM 2013).

Table 2. Changes to aqueous and sediment chemistry observed a the completion of the 28-day bioturbation bioassay

Sediment	Bioturbation Intensity	TSS (mg L^{-1})	Overlying Water Concentration (µg L^{-1})				Profile	AVS (µmol/g)	Dilute-acid extractable metals (AEM) (mg kg^{-1}, dry weight)			
			Fe	Mn	Cu	Zn			Fe	Mn	Cu	Zn
S1							Initial	0.1 ± 0.1	0.3 ± 0.001	33 ± 6	7.0 ± 0.4	7.0 ± 0.4
	No	25 ± 17	<2	4.0 ± 6.0	1.1 ± 1.0	<0.5	Surface	1 ± 0.5	0.3 ± 0.02	26 ± 3	9.0 ± 0.4	28 ± 0.8
							Deeper	2 ± 1	0.3 ± 0.1	27 ± 3	7 .0 ± 1.0	24 ± 5
	Low	34 ± 11	3 ± 5	12 ± 9	1.2 ± 0.4	0.5 ± 1	Surface	0.3 ± 0.5	0.3 ± 0.002	43 ± 26	10 ± 1	30 ± 0.5
							Deeper	2 ± 1	0.3 ± 0.001	24 ± 0.3	8.0 ± 1	29 ± 0.4
	High	93 ± 34	2 ± 3	31 ± 25	<0.8	1.0 ± 1	Surface	0.3 ± 0.5	0.3 ± 0.2	27 ± 12	9.0 ± 5.2	48 ± 28
							Deeper	2 ± 1	0.1 ± 0.1	10 ± 4	3.2 ± 3.0	19 ± 7
S2							Initial	0.2± 0.1	1 ± 0.07	110 ± 14	240 ± 20	560 ± 30
	No	15 ± 11	3 ± 8	0.4 ± 2	7.4 ± 3.0	9.3 ± 4.1	Surface	1 ± 0.3	1 ± 0.1	77 ± 14	160 ± 20	340 ± 50
							Deeper	0.5 ± 0.2	1 ± 1	81 ± 5	140 ± 5	380 ± 20
	Low	20 ± 16	2 ± 9	18 ± 18	6.2 ± 3	5.4 ± 2.2	Surface	1 ± 0.1	2 ± 0.1	190 ± 34	280 ± 12	610 ± 20
							Deeper	1 ± 0.3	1 ± 0.03	130 ± 2	240 ± 10	570 ± 40
	High	190 ± 30	4 ± 12	96 ± 55	4.0 ± 1.2	5.3 ± 2.0	Surface	1 ± 0.5	2 ± 1	230 ± 40	500 ± 110	1200 ± 360
							Deeper	0.5 ± 0.2	1 ± 0.1	130 ± 9	280 ± 30	690 ± 60
S3							Initial	10± 2	0.4 ± 0.06	25 ± 3	4.0 ± 2	810 ± 70
	No	17 ± 7	2 ± 3	1.2 ± 1.3	15 ± 3.4	76 ± 12	Surface	14 ± 2	0.5 ± 0.02	30 ± 8	58 ± 58	1100 ± 300
							Deeper	15 ± 2	0.5 ± 0.02	28 ± 3	3 ± 2	1400 ± 580
	Low	18 ± 12	4 ± 5	11 ± 5	9.1 ± 3.3	190 ± 50	Surface	4 ± 3	0.5 ± 0.030	27 ± 12	6 ± 4	800 ± 60
							Deeper	11 ± 3	0.5 ± 0.02	25 ± 1	1.0 ± 0.4	860 ± 50
	High	65 ± 30	3 ± 2	49 ± 15	0.8 ± 0.5	290 ± 140	Surface	3 ± 2	0.5 ± 0.06	33 ± 15	160 ± 120	900 ± 120
							Deeper	8 ± 1	0.5 ± 0.04	24 ± 3	1.0 ± 1.0	740 ± 50

* TSS refers to total suspended solids (*n=3*). AVS = acid-volatile sulfide. Mean ± standard deviation (*n=15*) of dissolved metals in overlying water over 28 days. Concentrations of AVS and AEM are for the initial (bulk sediment) and the concentrations after 28 days in surface and deeper sediment

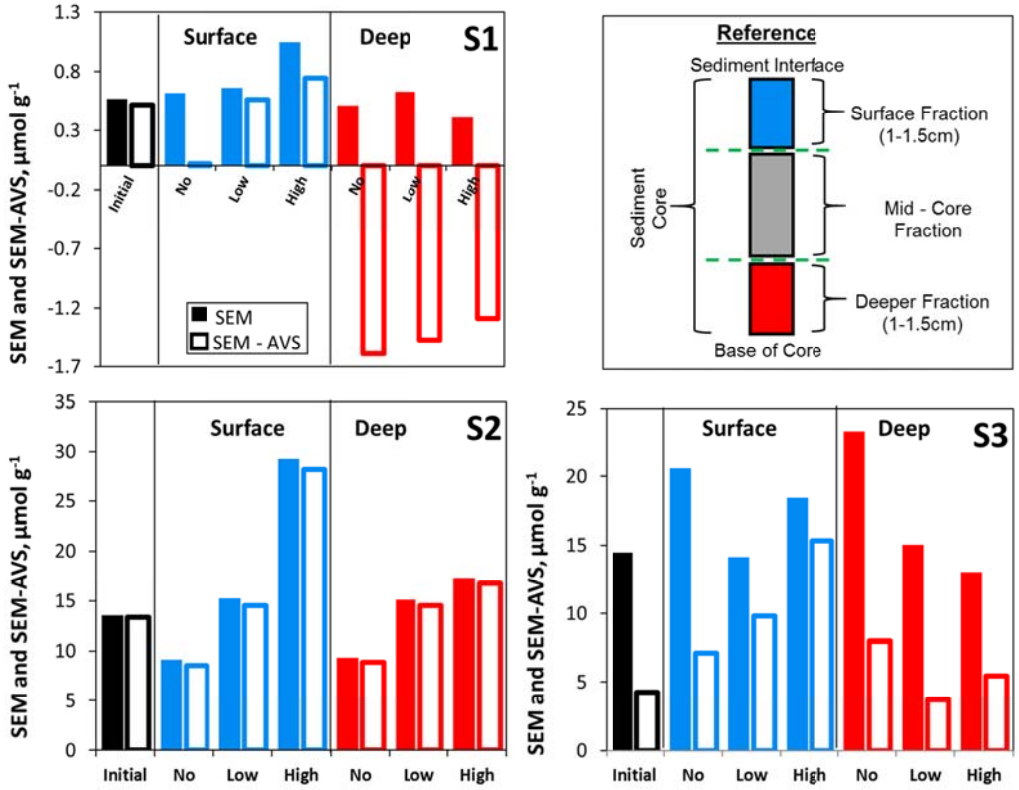

Figure 1. Bioturbation-induced changes in SEM-AVS during the 28 day bioassay for each test sediment. *No* refers to sediments with no organisms, *Low* refers to treatments with low bioturbation (bivalves only) and *High* refers to sediments with high levels of bioturbation (bivalves with amphipods).

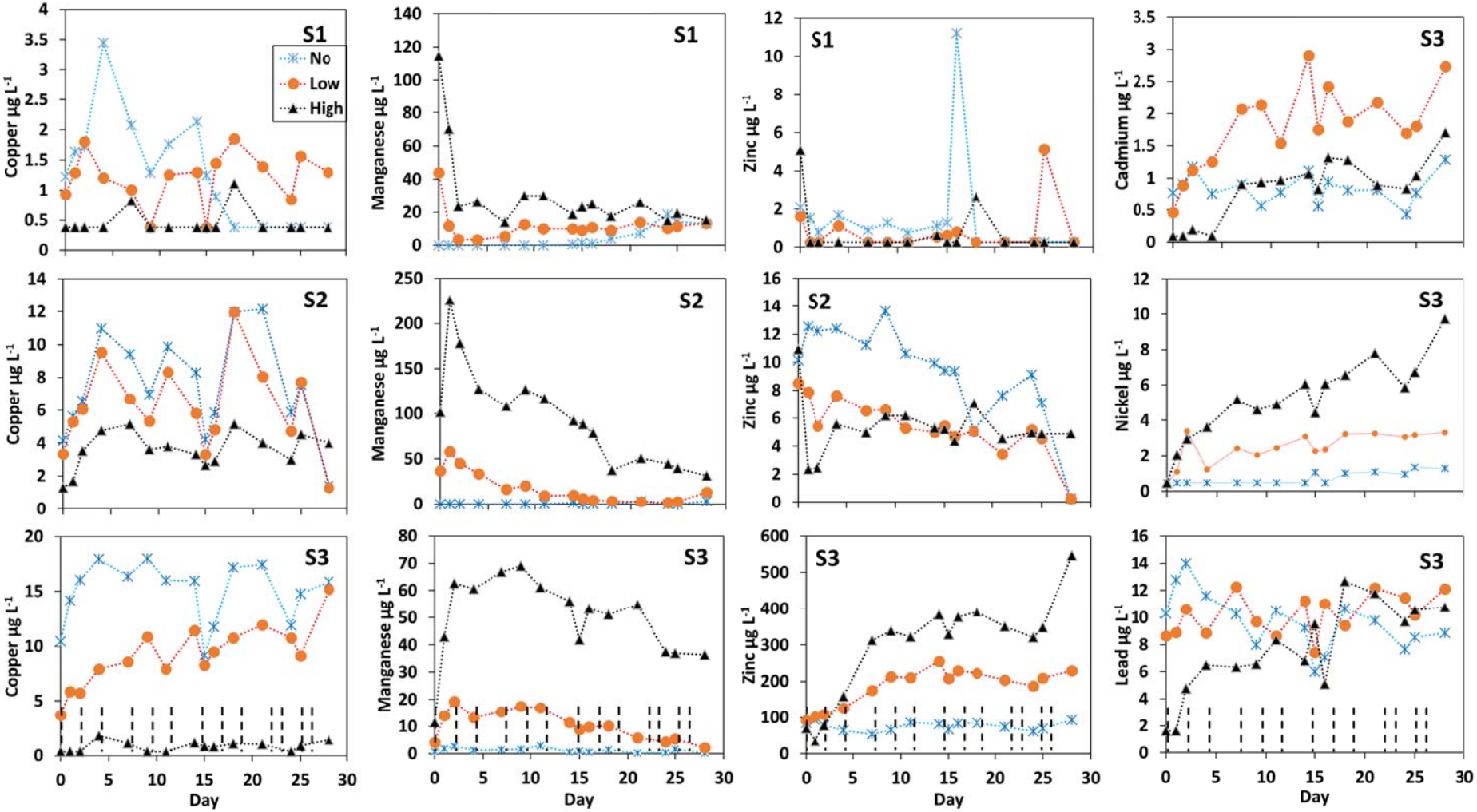

Figure 2. Dissolved metal concentrations for Cu, Mn, Zn (S1, S2 and S3) and Cd, Ni and Pb (S3) over the 28-d experiment. Black dotted lines in the bottom graphs are indicative of water changes which were carried out during the test. Blue star markers are indicative of control sediments which contain no organisms (No). Round orange markers are indicative of concentrations for tests with Low bioturbation (only bivalves) and black triangles are indicative of concentrations for tests exposed to High bioturbation (bivalves and amphipods).

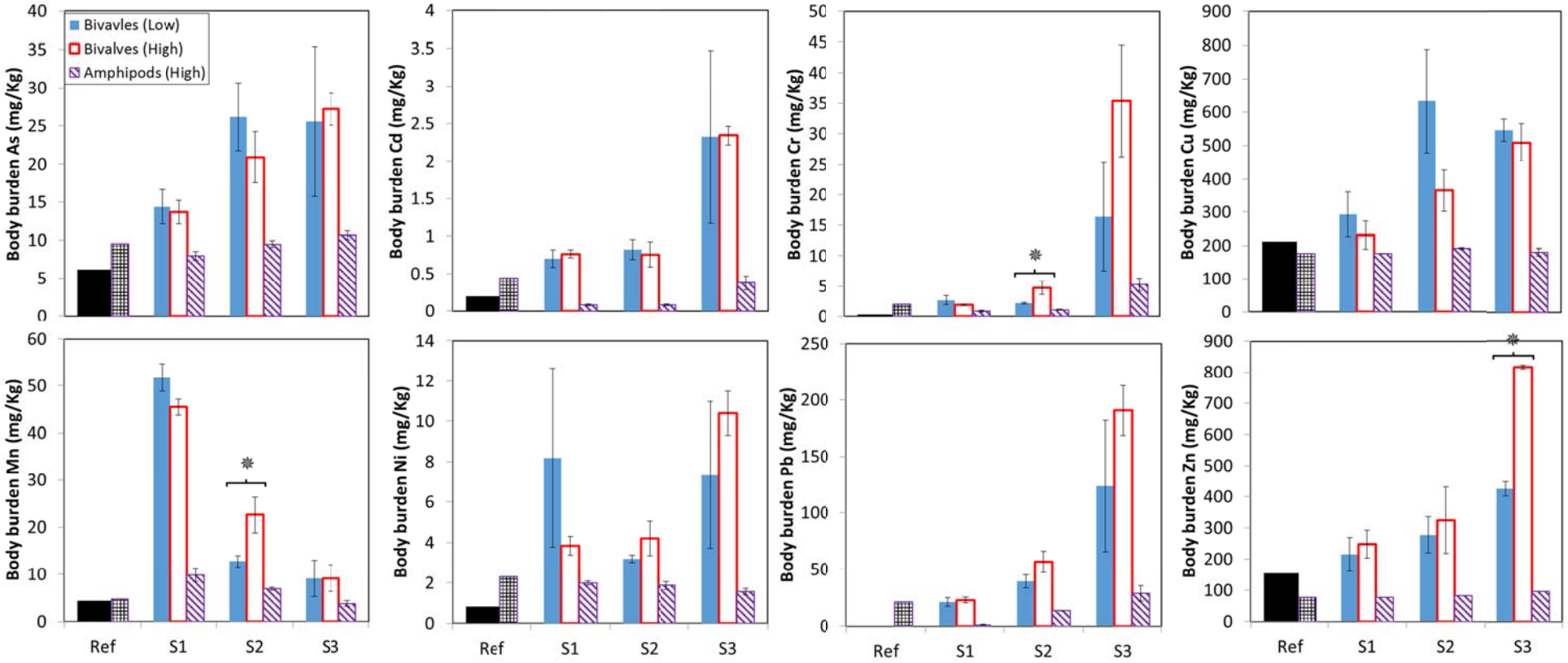

Figure 3. Organism bioaccumulation during the experiment (mean ± SE), for bivalves, n=15 (5 bivalves per replicate, 3 replicates per treatment). For amphipods, n=18 (6 amphipods per replicate, 3 replicates per treatment) (See Supplementary Table A2). Untreated (reference) bivalves are represented by black (■) columns and untreated amphipods are represented by hatched (▦) columns. A ❋ has been assigned to identify significant differences between high and low bioturbation.

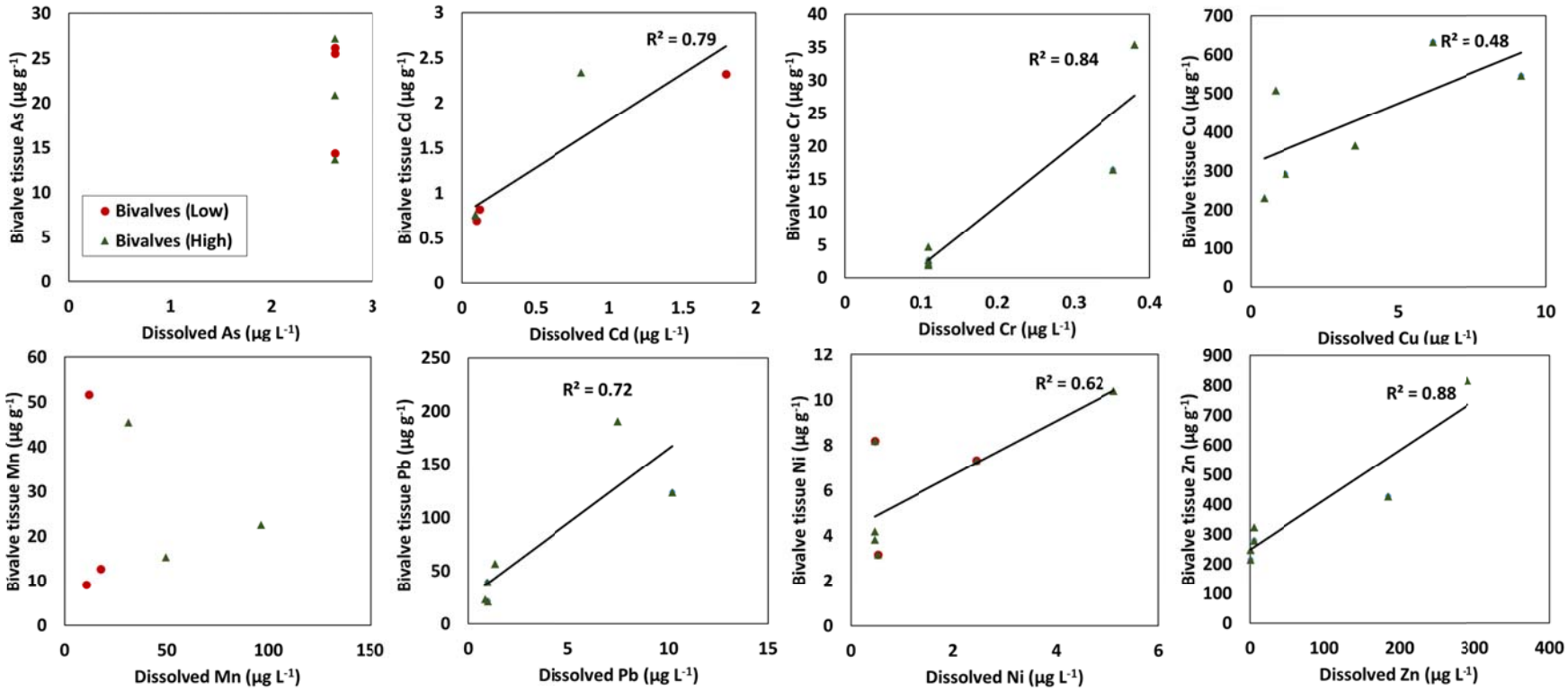

Figure 4. Comparison between average metal tissue concentrations in bivalves with average dissolved metal concentrations in the overlying waters. Note: linear regressions are only shown for data where the value for R^2 is ≥ 0.5.

Supplementary Information for paper

The impact of single and multiple sources of sediment bioturbation on the bioavailability and assessment of risks posed by metal contaminants to benthic marine organisms.

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Table A1 Physicochemical properties of initial sediments used for the 28-day bioturbation bioassay

Sediment	Al (%)	Fe (%)	Mn	Ag	As	Cd	Cr	Cu	Ni	Pb	V	Zn
						TRM (mg kg^{-1} or % for Al and Fe, dry weight)						
S1	0.3 ± 0.04	0.8 ± 0.1	44 ± 16	<0.1	2.1 ± 0.3	0.3 ± 0.1	5.0 ± 1	16 ± 2	2.3 ± 0.3	10 ± 1	17 ± 2	37 ± 4
S2	1.1 ± 0.1	4.0 ± 0.5	240 ± 90	1.0 ± 0.4	40 ± 2*	2 ± 0.2*	68 ± 1	550 ± 10+	19 ± 0.1	460 ± 6+	76 ± 0.2	900 ± 3+
S3	1.0 ± 0.04	0.5 ± 0.02	67 ± 2	5.0 ± 0.04+	50 ± 1*	8 ± 0.1*	390 ± 10+	1200 ± 30+	36 ± 0.7*	1400 ± 20+	51 ± 2	2800 ± 30+
						TRM of <63 µm sediment size fraction (mg kg^{-1} or % for Al and Fe, dry weight)						
S1	1 ± 0.05	2 ± 0.02	64 ± 2	<0.1	3 ± 0.2	1 ± 0.04	12 ± 0.4	44 ± 2	6 ± 0.2	15 ± 1	44 ± 1	92 ± 1
S2	1 ± 0.01	4 ± 0.04	130 ± 2	1 ± 0.1	33 ± 1*	2 ± 0.05*	66 ± 1	490 ± 30+	19 ± 0.5	400 ± 12*	74 ± 1	790 ± 31*
S3	1 ± 0.05	4 ± 0.1	180 ± 3	5 ± 0.04+	57 ± 1	10 ± 0.03*	450 ± 5+	1400 ± 4+	43 ± 1+	1500 ± 20*	62 ± 3	3300 ± 30*
						Dilute-acid extractable metals (AEM) (mg kg^{-1} or % for Al and Fe, dry weight)						
S1	0.1 ± 0.1	0.3 ± 0.09	33 ± 6	0.1 ± 0.03	0.5 ± 0.2	0.2 ± 0.05	1.0 ± 0.1	7.0 ± 0.4	1.0 ± 0.04	8.0 ± 0.2	30 ± 1	30 ± 1
S2	0.4 ± 0.1	1.0 ± 0.8	113 ± 14	0.2 ± 0.02	11 ± 1	1.0 ± 0.2	24 ± 2	240 ± 20*	5.6 ± 0.3	380 ± 20+	560 ± 30+	530 ± 50+
S3	0.3 ± 0.1	0.4 ± 0.06	25 ± 3	0.2 ± 0.01	1.0 ± 0.4	2.0 ± 0.2*	55 ± 6	0.4 ± 0.04	5.0 ± 0.5	390 ± 60+	850 ± 94+	820± 70+
						AEM/TRM Ratio						
S1	-	-	0.2	0	0.2	0.7	0.2	0.5	0.8	0.5	0.5	0.3
S2	-	-	0.3	0.2	0.3	0.5	0.3	0.4	0.8	0.4	0.4	0.6
S3	-	-	0.1	0.04	0.02	0.2	0.1	0.003	0.3	0.3	0.3	0.3
SQGV				*1*	*20*	*1.5*	*80*	*65*	*21*	*50*		*200*
SQGV – high				*3.7*	*70*	*10*	*370*	*270*	*52*	*220*		*410*

<63 µm (%) = percentage (by mass) of fine sediment particles; TRM (<63 µm) refers to TRM on the <63 µm sediment fraction. AEM = 1 M HCl extractable metals. Mean ± standard deviation. SQGV and SQGV-high are those upper guideline values as recommended in the revised ANZECC/ARMCANZ sediment quality guidelines (Simpson and Batley, 2013). * = exceed SQGV. + = exceed SQGV-high.

Table A2. Bioaccumulation effects and system chemistry

Sediment	Organism and Bioturbation Intensity	Survival (%)	*N*#	Total body burden (mg kg^{-1})							
				As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Initial	Bivalves	-	15	6 ± 1	0.2 ± 0.04	0.3 ± 0.1	210 ± 80	4 ± 5	1 ± 0.1	0.3 ± 0.01	150 ± 12
	Amphipods	-	18	9 ± 0.6	0.4 ± 0.04	2 ± 0.3	180 ± 20	5 ± 1	2 ± 0.2	21 ± 3	78 ± 3
S1	Bivalves/ Low Bio.	100	15	14 ± 4	1 ± 0.2	3 ± 1	290 ± 120	52 ± 5	8 ± 8	21 ± 7	210 ± 90
	Bivalves/ High Bio.	100	15	14 ± 3	1 ± 0.1	2 ± 0.2	230 ± 70	45 ± 3	4 ± 1	23 ± 4	250 ± 80
	Amphipods/ High Bio.	89	16	8 ± 1	0.1 ± 0.02	1 ± 0.2	170 ± 10	10 ± 2	2 ± 0.2	1 ± 1	80 ± 1
S2	Bivalves/ Low Bio.	53	9	26 ± 8	1 ± 0.2	2 ± 0.2	630 ± 270	13 ± 2	3 ± 0.3	39 ± 10	280 ± 100
	Bivalves/ High Bio.	100	15	21 ± 6	1 ± 0.3	5 ± 1	350 ± 0.4	23 ± 3	4 ± 1	57 ± 16	320 ± 180
	Amphipods/ High Bio.	83	15	9.5 ± 1	0.1 ± 0.02	1 ± 0.1	190 ± 10	7 ± 1	2 ± 0.3	13 ± 0.3	83 ± 4
S3	Bivalves/ Low Bio.	87	13	26 ± 17	2 ± 2	16 ± 15	540 ± 60	9 ± 7	7 ± 6	120 ± 100	420 ± 40
	Bivalves/ High Bio.	100	15	27 ± 4	2 ± 0.2	35 ± 16	510 ± 100	15 ± 5	10 ± 2	190 ± 40	820 ± 11
	Amphipods/ High Bio.	94	17	11 ± 1	0.4 ± 0.2	5 ± 2	180 ± 20	4 ± 1	2 ± 0.3	29 ± 12	98 ± 19
		TSS (mg/L)		Dissolved metals in overlying water (µg/L)							
S1	No	25 ± 17		<5	<5	<0.2	<0.2	1 ± 1	1 ± 3	<3	<0.5
	Low	34 ± 11		<5	<0.2	<0.2	1 ± 0.4	12 ± 9	<0.9	<3	1 ± 1
	High	93 ± 34		<5	<0.2	<0.2	<0.8	31 ± 25	<0.9	<3	1 ± 1
S2	No	15 ± 11		<5	<5	<0.2	<0.2	7 ± 3	<0.9	<3	9 ± 4
	Low	20 ± 16		<5	<0.2	<0.2	6 ± 3	18 ± 18	<0.9	<3	5 ± 2
	High	190 ± 30		<5	1 ± 0.2	<0.2	4 ± 1	96 ± 55	<0.9	<3	5 ± 2
S3	No	17 ± 7		<5	<5	2 ± 1	0.2 ± 0.1	15 ± 3	<0.9	10 ± 2	76 ± 12
	Low	18 ± 12		<5	1 ± 0.5	0.3 ± 1	9 ± 3	11 ± 5	2 ± 1	10 ± 1	190 ± 50
	High	65 ± 30		<5	0.5 ± 0.1	0.3 ± 0.5	0.8 ± 0.5	49 ± 15	2 ± 1	7 ± 3	290 ± 140

For bioaccumulation results (Mean ± standard error (SE, n=3) (bivalves = 5 organisms per replicate; Biv and Amp = 5 bivalves and 6 amphipods per replicate). Survival % is based on the total number of organisms recovered from each condition at the end of the test (15 bivalves per test and 18 amphipods in each test containing both amphipods and bivalves). N# is the total number of organisms used in the digestion. TRM= total recoverable metals, TSS= total suspended solids.

Table A3. Overlying water chemistry during the 28 day bioassay

Sediment	Bioturbation Intensity	TSS (mg L^{-1})	Dissolved Metal Concentration (µg L^{-1})									
			Fe	Mn	As	Cd	Cr	Cu	Ni	Pb	V	Zn
S1	NO	25 ± 17	<2	4 ± 6	<5	<0.2	<0.2	1 ± 1	1 ± 3	<3	<0.9	<0.5
	Low	34 ± 11	3 ± 5	12 ± 9	<5	<0.2	<0.2	1 ± 0.4	<0.9	<3	<0.9	1 ± 1
	High	93 ± 34	2 ± 3	31 ± 25	<5	<0.2	<0.2	<0.8	<0.9	<3	<0.9	1 ± 1
S2	NO	15 ± 11	3 ± 8	0.4 ± 2	<5	<0.2	<0.2	7 ± 3	<0.9	<3	<0.9	9 ± 4
	Low	20 ± 16	2 ± 9	18 ± 18	<5	<0.2	<0.2	6 ± 3	<0.9	<3	<0.9	5 ± 2
	High	190 ± 30	4 ± 12	96 ± 55	<5	1 ± 0.2	<0.2	4 ± 1	<0.9	<3	1 ± 1	5 ± 2
S3	NO	17 ± 7	2 ± 3	1 ± 1	<5	2 ± 1	0.2 ± 0.1	15 ± 3	<0.9	10 ± 2	<0.9	76 ± 12
	Low	18 ± 12	4 ± 5	11 ± 5	<5	1 ± 0.5	0.3 ± 1	9 ± 3	2 ± 1	10 ± 1	<0.9	190 ± 50
	High	65 ± 30	3 ± 2	49 ± 15	<5	0.5 ± 0.1	0.3 ± 0.5	0.8 ± 0.5	5 ± 2	7 ± 3	<0.9	290 ± 140

* TSS refers to total suspended solids (*n=3*). For dissolved metals, *n=15* over 28 days. Mean ± standard deviation.

Table A4. General averaged water quality parameters

Sediment	Bioturbation Intensity	pH	DO	Salinity (ppt)	T (°C)
S1	No	7.8 ± 0.2	92 ± 0.4	35 ± 1	21 ± 1
	Low	8.1 ± 0.0	91 ± 0.4	35 ± 1	21 ± 1
	High	8.0 ± 0.1	93 ± 0.3	35 ± 1	21 ± 1
S2	No	8.1 ± 0.0	93 ± 0.4	35 ± 0.4	21 ± 1
	Low	8.1 ± 0.1	92 ± 0.4	34 ± 1	22 ± 1
	High	8.0 ± 0.1	90 ± 0.5	34 ± 2	22 ± 1
S3	No	8.1 ± 0.0	92 ± 0.4	35 ± 0.5	21 ± 1
	Low	8.1 ± 0.1	92 ± 0.4	35 ± 1	21± 1
	High	7.9 ± 0.1	91 ± 0.3	35 ± 1	22 ± 1

* DO_2 refers to dissolved oxygen. For all test parameters, *n=4,* taken weekly over 28 days. Mean ± standard deviation.

Table A5. Chemistry of sediments after 28 day bioassay

Sediment	Strata	Bioturbation Intensity	SEM	AVS	SEM-AVS	Dilute-acid extractable metals (AEM) (mg kg^{-1}, dry weight)									
				(µmol g^{-1})		Fe (%)	Mn	As	Cd	Cr	Cu	Ni	Pb	V	Zn
	Initial	-	0.6	0.1	0.5	0.3 ± 0.01	33 ± 6	0.5 ± 0.2	0.2 ± 0.05	1.0 ± 0.1	7.0 ± 0.4	1.0 ± 0.04	8.0 ± 0.2	30 ± 1	30 ± 1
S1	Surface	No	0.6	0.6	0.01	0.3 ± 0.02	26 ± 3	0.5 ± 0.1	0.4 ± 0.1	1 ± 0.05	9 ± 0.5	0.5 ± 0.1	10 ± 0.5	10 ± 0.2	28 ± 0.8
	Surface	Low	0.7	0.1	1	0.3 ± 0.02	43 ± 26	1 ± 0.4	0.4 ± 0.1	1 ± 0.01	10 ± 1	0.5 ± 0.1	9 ± 1	9 ± 1	30 ± 0.5
	Surface	High	1	0.3	1	0.3 ± 0.2	27 ± 12	1 ± 0.4	0.8 ± 0.3	1 ± 1	9 ± 5	1 ± 0.3	10 ± 5	10 ± 5	48 ± 28
	Deeper	No	0.5	2	-2	0.3 ± 0.1	27 ± 3	0.5 ± 0.2	0.4 ± 0.1	1 ± 0.3	7 ± 1	0.3 ± 0.2	8± 2	8 ± 2	24 ± 5
	Deeper	Low	0.6	2	-1	0.3 ± 0.01	24 ± 0.3	1 ± 0.02	0.4 ± 0.02	1 ± 0.03	8 ± 1	1 ± 0.1	10 ± 1	10 ± 0.3	29 ± 0.4
	Deeper	High	0.4	2	-1	0.1 ± 0.1	10 ± 4	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	3 ± 3	0.2 ± 0.1	4 ± 2	4 ± 2	19 ± 7
	Initial	-	13.5	0.2	13.3	1 ± 0.07	113 ± 14	11 ± 1	1.0 ± 0.2	24 ± 2	240 ± 20*	5.6 ± 0.3	380 ± 20+	560 ± 30+	530 ± 50+
S2	Surface	No	9	0.6	9	1 ± 0.1	77 ± 14	8 ± 2	1 ± 0.3	19 ± 3	160 ± 20	4 ± 1	270 ± 40	23 ± 4	340 ± 50
	Surface	Low	15	0.7	15	2 ± 0.1	190 ± 34	20 ± 1	2 ± 1	29 ± 3	280 ± 12	6 ± 1	420 ± 30	35 ± 2	610 ± 20
	Surface	High	29	1	28	2 ± 1	230 ± 40	31 ± 7	3 ± 1	53 ± 12	500 ± 110	12 ± 2	740 ± 210	65 ± 15	1160 ± 360
	Deeper	No	9	0.5	9	1 ± 1	81 ± 5	7 ± 1	1 ± 0.4	18 ± 1	140 ± 5	4 ± 0.2	270 ± 10	24 ± 2	380 ± 20
	Deeper	Low	15	0.6	15	1 ± 0.03	130 ± 2	12 ± 2	1 ± 0.2	31 ± 1	240 ± 10	6 ± 0.03	440 ± 30	39 ± 1	570 ± 40
	Deeper	High	17	0.5	17	1 ± 0.1	130 ± 9	15 ± 2	1 ± 1	31 ± 3	280 ± 30	6 ± 0.5	470 ± 50	38 ± 3	690 ± 60
	Initial	-	14.4	10	4.3	0.4 ± 0.06	25 ± 3	1.0 ± 0.4	2.0 ± 0.2*	55 ± 6	0.4 ± 0.04	5.0 ± 0.5	390 ± 60+	850 ± 94+	820± 70+
S3	Surface	No	21	14	7	0.5 ± 0.02	30 ± 8	2 ± 2	4 ± 2	63 ± 3	58 ± 58	6 ± 1	460 ± 50	15 ± 1	1140 ± 300
	Surface	Low	14	4	10	0.5 ± 0.03	27 ± 12	1 ± 0.3	2 ± 0.2	62 ± 2	6 ± 4	6 ± 1	420 ± 40	16 ± 0.4	800 ± 60
	Surface	High	18	3	15	0.5 ± 0.06	33 ± 15	5 ± 3	3 ± 1	63 ± 14	160 ± 120	7 ± 1	420 ± 110	19 ± 4	900 ± 120
	Deeper	No	23	15	8	0.5 ± 0.02	28 ± 3	0.4 ± 0.1	5 ± 4	67 ± 3	3 ± 2	6 ± 1	450 ± 20	17 ± 0.2	1370 ± 580
	Deeper	Low	15	11	4	0.5 ± 0.02	25 ± 1	1 ± 1	2 ± 0.3	67 ± 2	1 ± 0.4	6 ± 1	420 ± 60	17 ± 0.4	860 ± 50
	Deeper	High	13	8	5	0.5 ± 0.04	24 ± 3	0.5 ± 0.1	2 ± 0.1	16 ± 6	1 ± 1	5 ± 1	390 ± 20	15 ± 1	740 ± 50

AEM = 1 M HCl extractalbe metals. SEM- simultaneously extractable metals in 1M HCl. AVS= acid volatile sulfide, the reactive sulfide fraction of sediments. SEM-AVS = the theoretical fraction of metals bioavailable to the benthos. Mean ± standard deviation.

Table A6. Chemistry of sediments after 28 day bioassay(2)

Sediment	Bioturbation Intensity	Profile	SEM ($\mu mol\ g^{-1}$)	AVS ($\mu mol\ g^{-1}$)	SEM-AVS ($\mu mol\ g^{-1}$)	SEM ($\mu mol\ g^{-1}$, dry weight)						
						Fe	Mn	Cd	Cu	Ni	Pb	Zn
S1	No	Surface	0.6	0.6	0.01	50	0.001	0.001	0.1	0.01	0.04	0.4
		Deeper	0.5	2	-2	50	0.001	0.001	0.1	0.01	0.04	0.4
	Low	Surface	0.7	0.1	1	50	0.002	0.002	0.2	0.01	0.04	0.5
		Deeper	0.6	2	-1	50	0.001	0.001	0.1	0.01	0.04	0.4
	High	Surface	1	0.3	1	90	0.002	0.002	0.2	0.02	0.1	0.7
		Deeper	0.4	2	-1	40	0.001	0.001	0.1	0.005	0.03	0.3
S2	No	Surface	9	0.6	9	140	0.004	0.004	2.4	0.1	1.2	5.4
		Deeper	9	0.5	9	150	0.01	0.01	2.2	0.1	1.3	5.8
	Low	Surface	15	0.7	15	260	0.01	0.01	4.4	0.1	1.9	8.8
		Deeper	15	0.6	15	250	0.01	0.01	3.6	0.1	2.1	9.3
	High	Surface	29	1	28	420	0.03	0.03	7.7	0.2	3.5	18
		Deeper	17	0.5	17	450	0.02	0.02	4.3	0.1	2.2	11
S3	No	Surface	21	14	7	80	0.03	0.03	1.0	0.1	2.2	18
		Deeper	23	15	8	90	0.05	0.05	0.04	0.1	2.1	21
	Low	Surface	14	4	10	74	0.02	0.02	0.08	0.1	1.8	12
		Deeper	15	11	4	80	0.02	0.02	0.01	0.1	1.8	13
	High	Surface	18	3	15	80	0.02	0.02	2.2	0.1	2.2	14
		Deeper	13	8	5	70	0.01	0.01	0.01	0.1	1.6	11

AEM = 1 M HCl extractable metals. SEM- simultaneously extractable metals in 1M HCl. AVS= acid volatile sulfide, the reactive sulfide fraction of sediments. SEM-AVS = the theoretical fraction of metals bioavailable to the benthos. Mean ± standard deviation.

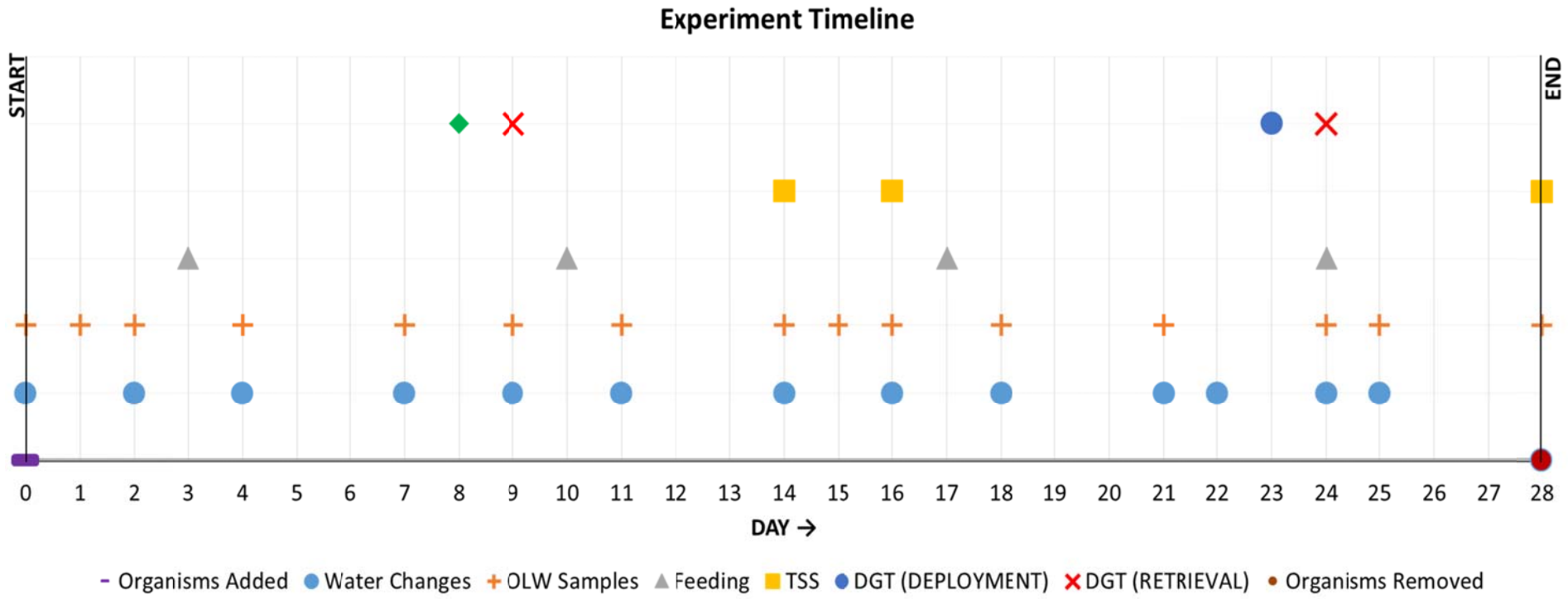

Figure A1. Test Timeline.

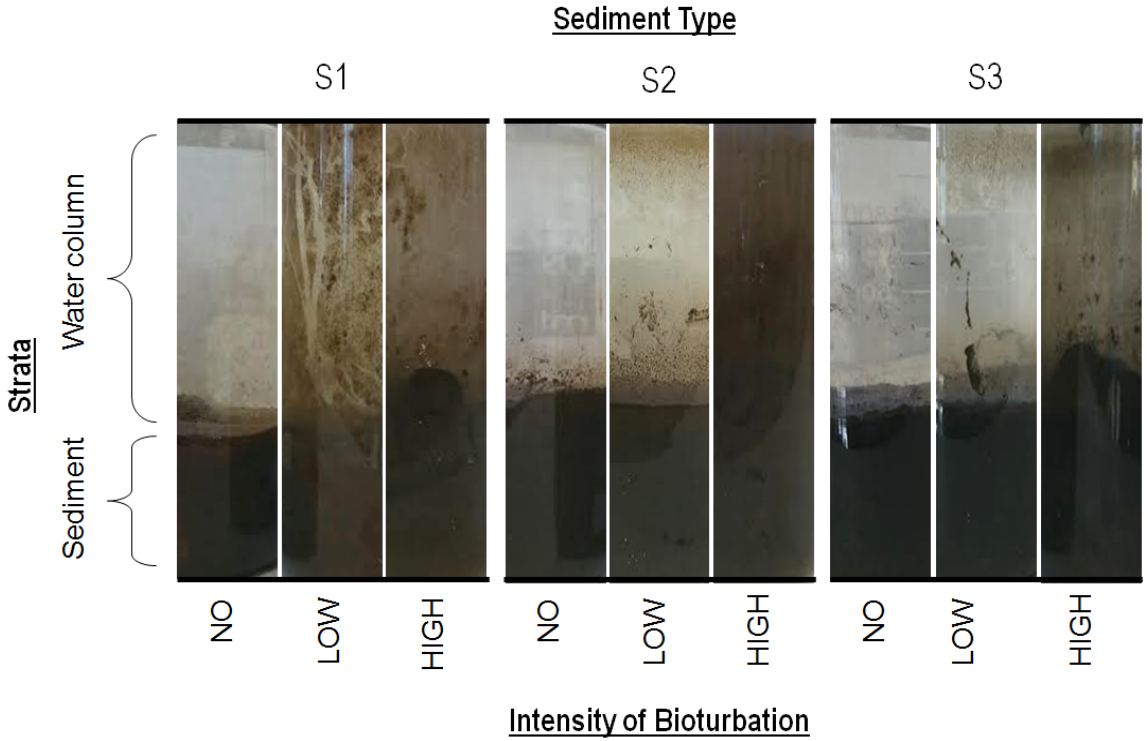

Figure A2. Visual representation of the impact bioturbation on the concentration of total suspended solids in the overlying water for the three test conditions

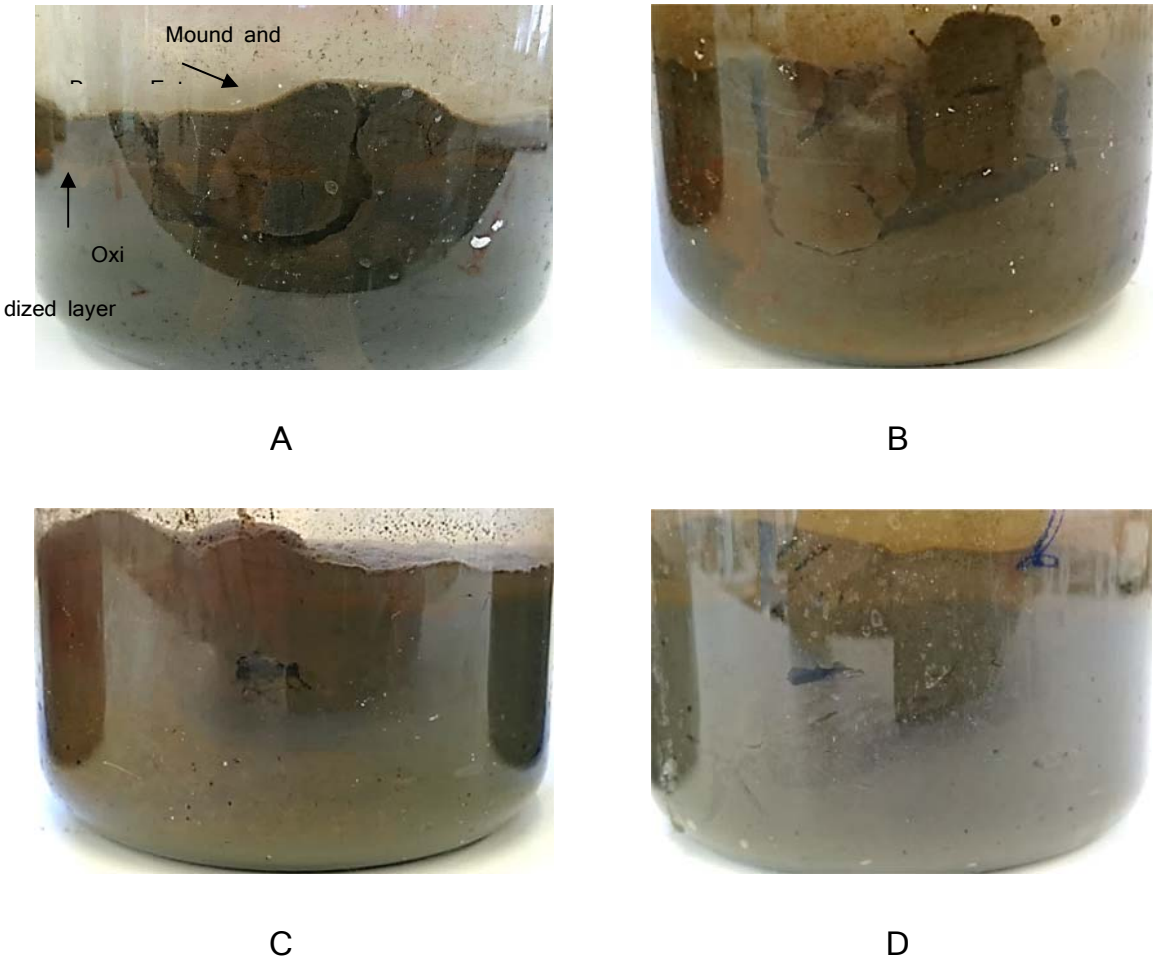

Figure A3. Visual representation of sediment characteristics during bioturbation. A= U-shaped amphipod burrow with entrance mound (S1), B= Y-shaped amphipod burrow and associated networks (S2), C & D= bivalve dens (S2 (C) and S3 (D)).

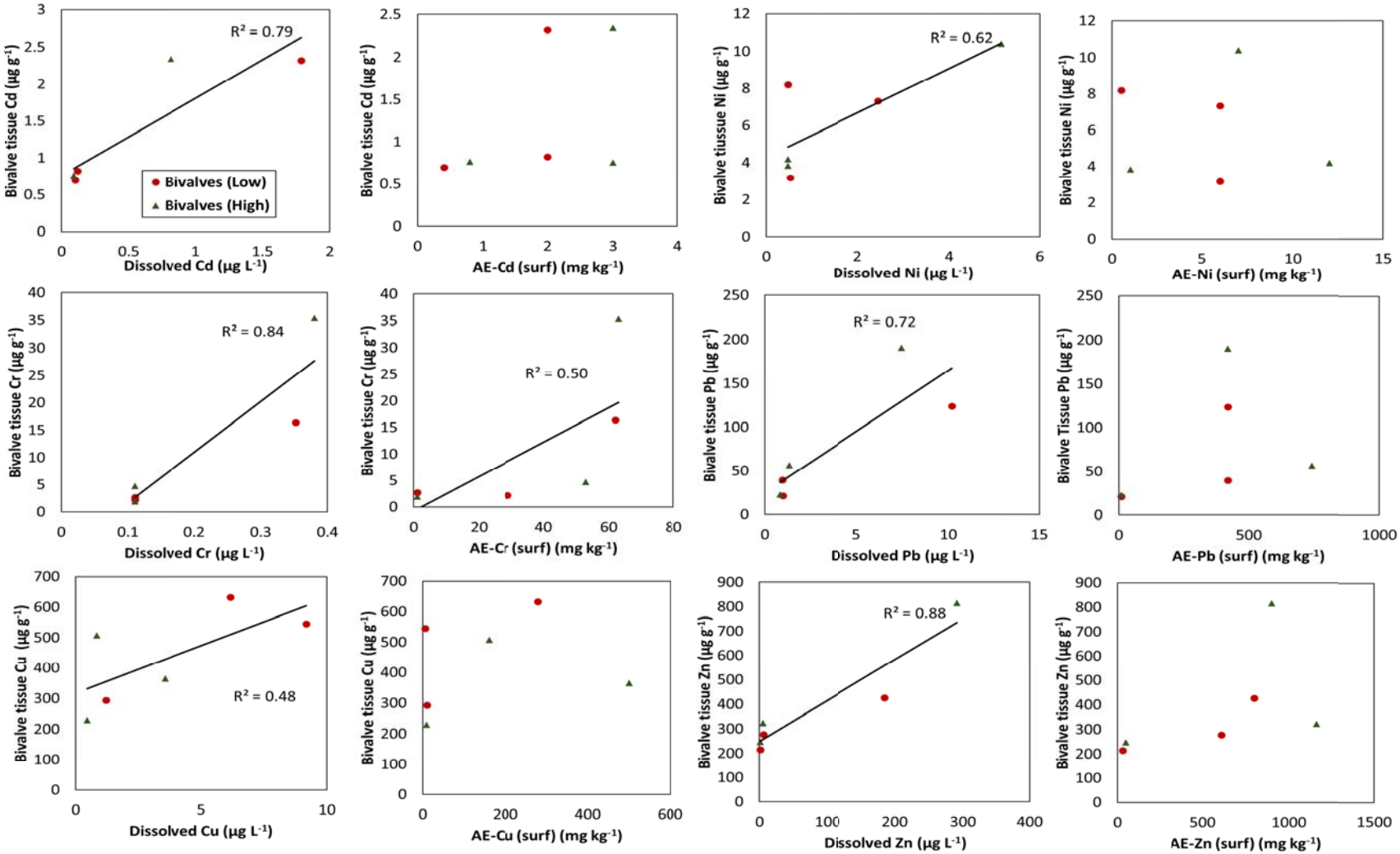

Figure A4. Comparisons between average metal concentrations in bivalve tissues with average metal concentrations in the dissolved and solid phase (Cr, Cu, Ni, Pb, Zn). Note: linear regressions are only shown for data where the value for R^2 is ≥ 0.5.

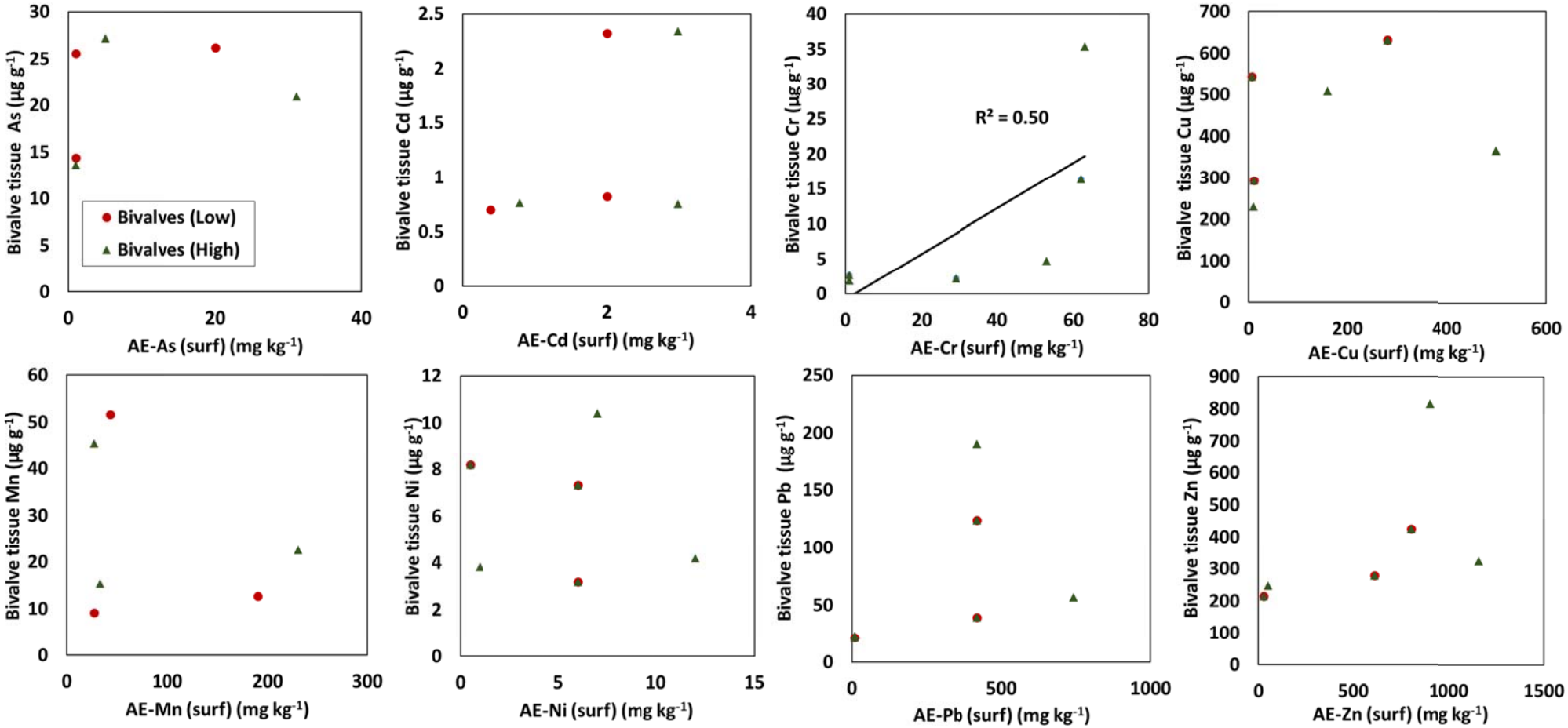

Figure A5. Comparisons between average metal tissue concentrations in bivalves with average dilute-acid extractable metal concentrations in surficial sediments. Note: linear regressions are only shown for data where the value for R^2 is ≥ 0.5.

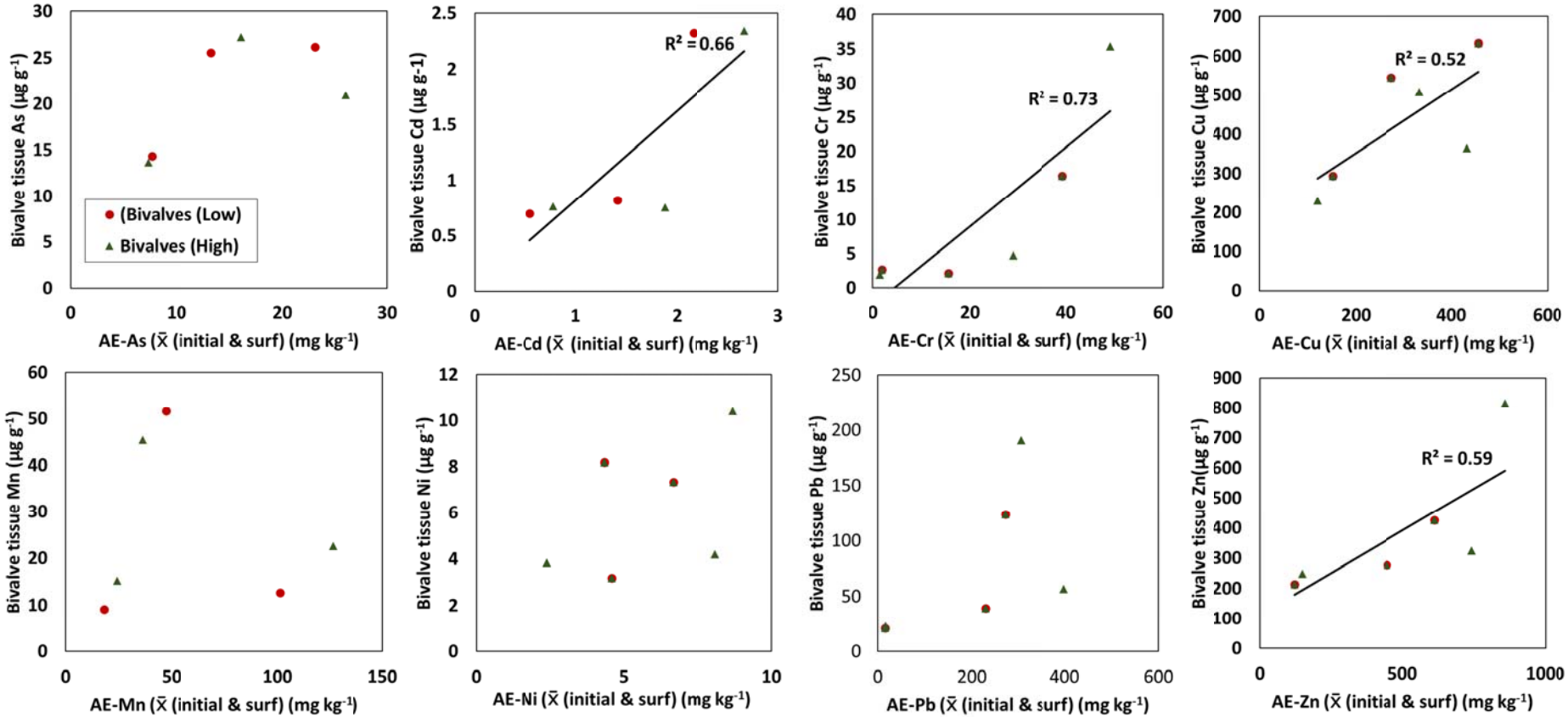

Figure A6. Comparisons between average metal tissue concentrations in bivalves with average dilute-acid extractable metal concentrations in surficial sediments as a function of initial AEM concentrations ($AEM_{initial}$ + $AEM_{surface}$)/2).